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Deciphering Circuit-level Mechanisms Underlying Intrinsic Epileptogenicity of Cortical Tubers in TSC

**PRINCIPAL INVESTIGATOR:**
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**CONTRACTING ORGANIZATION:**
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Fort Detrick, Maryland 21702-5012

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**5d. PROJECT NUMBER**
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**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

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Houston TX 77030, USA

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Development Command
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**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT: Cortical tuber (CT), the neuropathological hallmark of tuberous sclerosis complex (TSC), is a major type of pediatric cortical dysplasia characterized by cortical dyslamination and the presence of dysplastic neurons such as balloon cells and cytomegalic neurons. It is generally believed that seizures associated with TSC arise from CTs, and preoperative localization and subsequent surgical removal of epileptogenic CTs often results in seizure freedom. A critical and unsolved question is what circuit components are present in CT that underlie its intrinsic epileptogenicity. While dysplastic cell types in CTs could be plausible culprits for seizure generation, we currently know little about how these abnormal cell types connect to each other and to more normal cell types to drive seizure activity. To this end, we are taking advantage of the wealth of epileptogenic CTs surgically resected at our institute, and propose to apply a high-throughput multipatch recording method (up to 12 cells) into brain slices prepared from these CTs to interrogate the connection abnormalities of CT local circuit. Massive, multi-patch recordings of distinct cell types from the same CT slices will enable unraveling the specific connectivity patterns of abnormal cell types characteristic of CTs, in addition to their intrinsic electrophysiological properties. The study will provide the first insights into circuit-level mechanisms underlying CT epileptogenesis, with the potential to identify the potential "epileptic" cell types.**
**Once we identify candidates for the "epileptic" cell type, we will use a novel single-cell RNA-sequencing technique, Patch-seq, to derive their transcriptome to test if they have specific transcriptional profiles**

**15. SUBJECT TERMS**
Tuberous sclerosis complex, connectivity, electrophysiology, single-cell RNA-sequencing, balloon cells, cytomegalic neurons

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
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| Unclassified | Unclassified | Unclassified | Unclassified | 16 | USAMRMC |

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Tuberous sclerosis complex (TSC) is a complex genetic disorder often associated with intractable epilepsy, autism, and intellectual disability, characterized by the presence of focal cortical dysplasia known as cortical tubers (CTs) as well as tumors in other organs. While some exceptions may apply in certain cases, it is generally believed that seizures associated with TSC arise from CTs1-7, and preoperative localization and subsequent surgical removal of epileptogenic CTs often results in seizure freedom8-10The ability of cortical tubes present in CTs to generate ictal

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Tuberous sclerosis complex, connectivity, electrophysiology, single-cell RNA-sequencing, balloon cells, cytomegalic neurons

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

**What were the major goals of the project?**

*List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

| **Specific Aim 1 (specified in proposal):** Generate a morphological taxonomy of cell types in CTs and map their connections | **Timeline** | **% of completion** |
|---|---|---|
| **Major Task 1:** identifying the potential "epileptic" cell types by revealing the connectivity and intrinsic electrophysiological properties of major cell types in CTs (we need at least 25 TSC tissue cases and 5 control cases) | Months 1-17 | |
| Subtask 1: Simultaneous multi-cell patch recording on CT slices | 1-16 | ~80% |
| Subtask 2: Biocytin staining, NeuN staining, morphology reconstruction. | 2-16 | ~80% |
| Subtask 3: Data Analysis | 3-16 | ~65% |
| Milestone(s) Achieved: Revealing the connectivity pattern of major cell types in CT; Identifying the most likely candidate for "epileptic" cell types | 11-17 | ~80% |
| Local IRB/IACUC Approval | 0 | |
| | | |
| **Specific Aim 2:** Derive transcriptomic signatures of potential "epileptic" cell types in CTs using Patch-seq | 12-24 | |
| **Major Task 2:** to test if the potential "epileptic" cell types have specific transcriptional profiles (we need at least five TSC tissue cases and two control cases) | 12-24 | |
| Subtask 1: Patch-seq | 12-18 | ~25% |
| Subtask 2: Transcriptomic data analysis and Bioinformatics analysis | 13-24 | ~25% |
| | | |
| Subtask 3: Immunocytochemistry and in situ hybridization | 21-24 | ~10% |
| Milestone(s) Achieved: Mapping out the transcriptomic signatures of the potential "epileptic" cell types, such as cytomegalic interneurons. | 23-24 | ~15% |

**What was accomplished under these goals?**

*For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

**1. Electrophysiology and Connectivity analysis**

We have been continuing to recruit the TSC patients and control patients into this study for connectivity analysis. We collected fresh brain tissue samples from each enrolled patient, and the tissue was then sliced into the thin sections for electrophysiology and single-cell RNA-sequencing. For electrophysiological recordings and identification of synaptic connection, simultaneous whole-cell recordings of up to 12 neurons were obtained from brain slices as described previously to identify synaptic connections between cell pairs. The firing pattern of each recorded cell was recorded. Biocytin was injected into each neuron during recording sessions for the post hoc morphology recovery and identification. As stated in the previous report period, we have recorded and identified distinct cell types in the CTs by their morphology and electrophysiology criteria, including balloon cells, immature excitatory neurons at different developmental stages, cytomegalic excitatory neurons, and interneurons (see the previous report).

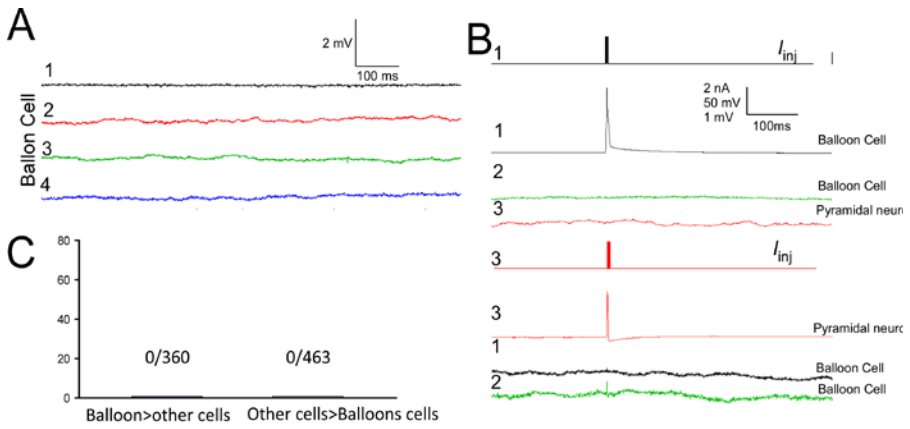


**Figure 1. Connectivity of Balloon cells.**
(A). Simultaneously recorded balloon cells from cortical tubes are synaptically quiet, suggesting no synaptic inputs to these cells. Note no synaptic events . (B) Stimulation of one balloon cell with current injections could not induce any synaptic events in nearby neurons. (C) The connectivity rate between balloon cells and other cells.

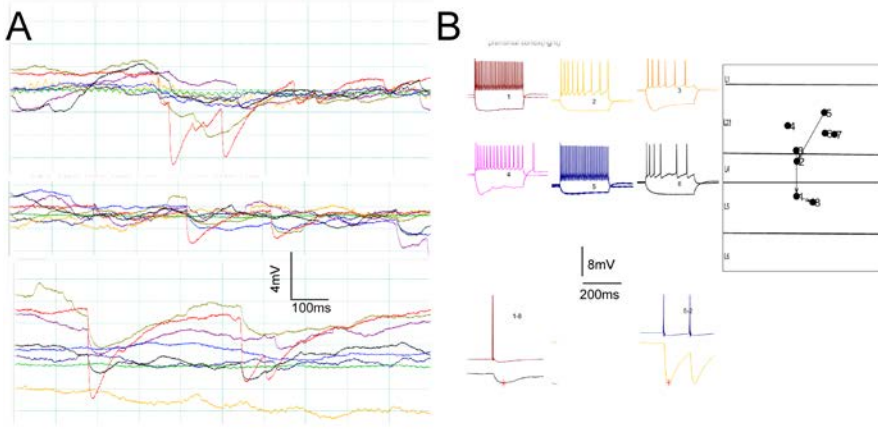


**Figure 2. GABAergic activity in the cortical tubes.**
A) In simultaneously recorded seven cells from CTs, all neurons except balloon cells received synchronous, robust GABA-mediated events.The traces with different colors were from the different cells.B) In simultaneously recorded six neurons from CT, single action potential evoked in the GABAergic interneurons (cell 1& 5, identified by firing patterns) could induce strong unitatory GABAergic postsynaptic events in excitatory neurons (cell 8$2, identified by firing pattern). No monosynaptic excitatory events could be evoked.

In this reporting period, we have collected more data to confirm our preliminary observations from the last reporting period on the connectivity patterns of different cell types in CTs. We confirmed that balloon cells do not form any synaptic connections with any cell types in CTs (Fig.1). We also confirmed that the relatively mature excitatory cells can contribute small, NMDA receptor-mediated excitatory synaptic events, while primitive excitatory neurons and cytomegalic excitatory neurons barely synapsed on any cell types, including interneurons (Figure not shown). Instead, GABAergic interneurons contribute to the vast majority of synaptic events in CT, and the CTs were dominated by robust synchronous GABA-mediated events (Fig 2A, B). These synchronous GABA-mediated events may arise from the spontaneous activity of cytomegalic interneurons (Fig.3). These giant interneurons have extensive axonal trees that expand across the whole depth of CT and project all cell types in CT (Fig.3). Importantly, these interneurons can fire as a rhythmic bursting activity (Fig.3D). Given the GABAergic action is depolarizing, not hyperpolarizing in CT (Talos et al., 2012), these interneurons may be "epileptic" cell types possibly acting as 'pacemakers' capable of spontaneous depolarizations that would drive local neurons and circuits into seizures (Cepeda et al., 2007). We have also collected the data from control tissue and suggested such unusual circuit components were CT-specific. We will continue to collect more data from control tissue to confirm the findings.

**2. Transcriptomic profiling of abnormal cell types in CT**

In the last six months, we have been redirecting our emphasis onto single-cell RNA-sequencing (scRNA-seq), aiming at identifying the transcriptomic signatures of each abnormal cell types, particularly cytomegalic interneurons, which may be the potential "epileptic" cell types essential for the CT epileptogenesis (see above). While we were still keeping using the Patch-seq approach as initially proposed, it should be noted that this approach is low yield and labor-intensive. We thus adopted another scRNA-seq protocol, 10 X Genomic droplet-based approach to facilitate our progress. The droplet-based scRNA-seq is a rapidly evolving, new technology which allows high-throughput genetic profiling of a large number of cells with an affordable budget and in a reasonable time period. Applying this technology in heterogeneous brain tissue like the human cortical tube allows the unbiased sequencing of tens of thousands of cells to reveal overall transcriptomic heterogeneity across all its constituent cell types. Rich datasets across all transcriptional heterogeneity provide a robust statistical power to identify the most specific genetic markers for each cell type. However, this approach is unable to provide any other phenotypic information for each cell type, including spatial location, morphological features, electrophysiological and functional properties. Patch-seq, on the other hand, can fill this gap by providing simultaneous access to a neuron's transcriptome, electrophysiology, and morphology. Patch-seq also allows RNA-seq of neurons of interest, for example, those that are fluorescently labeled or directly identified under light microscopy, such as balloon cells and cytomegalic cells in cortical tubes (CT, Figure 4). By combining the two protocols, droplet-based and Patch-seq, we thus can complement the strength of two distinct protocols to facilitate transcriptomic profiling of a cell type of high interest, such as balloon cell and cytomegalic cells (Figure 4).

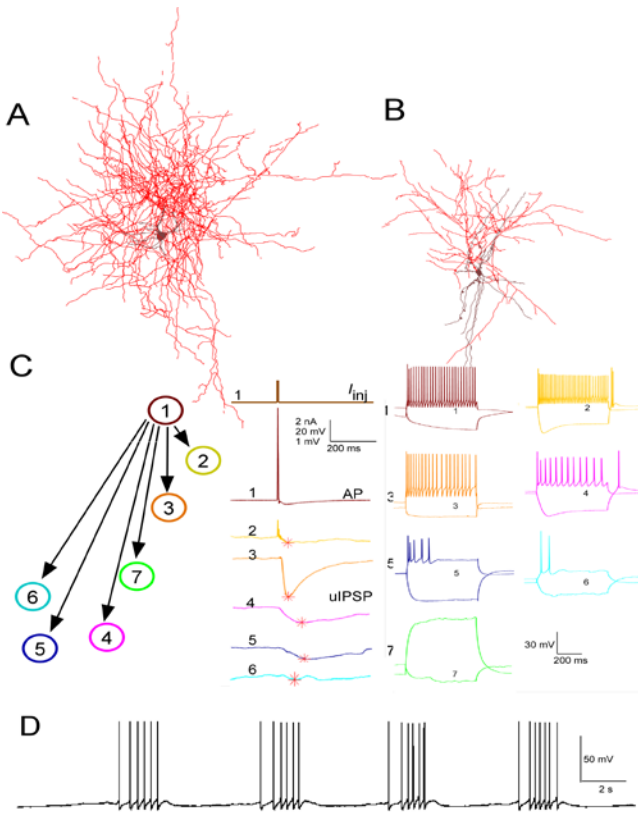


**Figure 3. Cytomegalic Interneurons may be major culprits for epileptogenicity.** The morphological recovery of cytomegalic interneurons in CT (A) and their counterpart in peritumor area (B).(C) In simultaneously recorded seven neurons from CT, single action potential evoked in cytomegalic interneuron (Cell 1) induced uIPSPs in all other cell types in CT. (D) Rhythmic bursting of a cytomegalic interneuron when the cell was recorded in resting membrance potentials.

***Tissue collection and dissociation for scRNA-seq*:** The tissue collection and dissociation procedures are modified from the existing protocol to adapt human brain tissue. Human cortical tissue is dissociated generally following the 10X Genomics Chromium sample preparation protocol. Briefly, the tissue is transferred to and incubated in 2 ml papain solution (Worthington, PAPL) at 34°C for 70 mins bubbled

with 95% O2/5% CO2. Samples are triturated with fire-polished Pasteur pipettes and passed through 40 µm cell strainers (Miltenyi Biotec). Cells are centrifuged for 5 mins at 300 rcf., and the supernatant is discarded. The dead cells and debris are removed by using the Dead Cell Removal Kit and Myelin Removal Kit (MACS). Cells are resuspended in 0.04% BSA solution and placed on ice for scRNA-seq.

***Single-cell RNA sequencing*:** Cells are counted and diluted in 1X PBS with 0.04% Bovine Serum Albumin (BSA) prior to loading onto the 10X Genomics Chromium instrument. Libraries are generated with the 10X Chromium Single Cell 3' v2 reagent kit according to the manufacturer's instructions and sequenced on an Illumina Nextseq500.

***Patch-seq in human brain slices*:** We have modified the patch-clamp recording protocol to improve RNA recovery (Cadwell et al., 2016; Cadwell et al., 2017). After 5-10 mins of the whole-cell recording of firing patterns and synaptic events of human cortical neurons in CTs, as well as dye filling and imaging (biocytin or fluorescent dyes) in human brain slices, RNA is collected from each neuron and complementary DNA (cDNA) is generated as described in detail in our previous study (Fig.4B) (Cadwell et al., 2016). Only high-quality cDNA samples (yield ≥ 2 ng, average length ≥ 1500 bp) are sequenced. After 18 amplification cycles, sequencing libraries are constructed from the cDNA using Tn5-mediated tagmentation (SMART-seq v4 (Clontech) (Picelli et al., 2014). Quality control is performed on both the amplified full-length cDNA and the library using an Agilent Bioanalyzer. cDNA libraries are sequenced using an Illumina HiSeq 3000 or 4000. Reads are aligned to the human genome using STAR (Dobin et al., 2013), normalized, and quantified as transcripts per million (TPM).

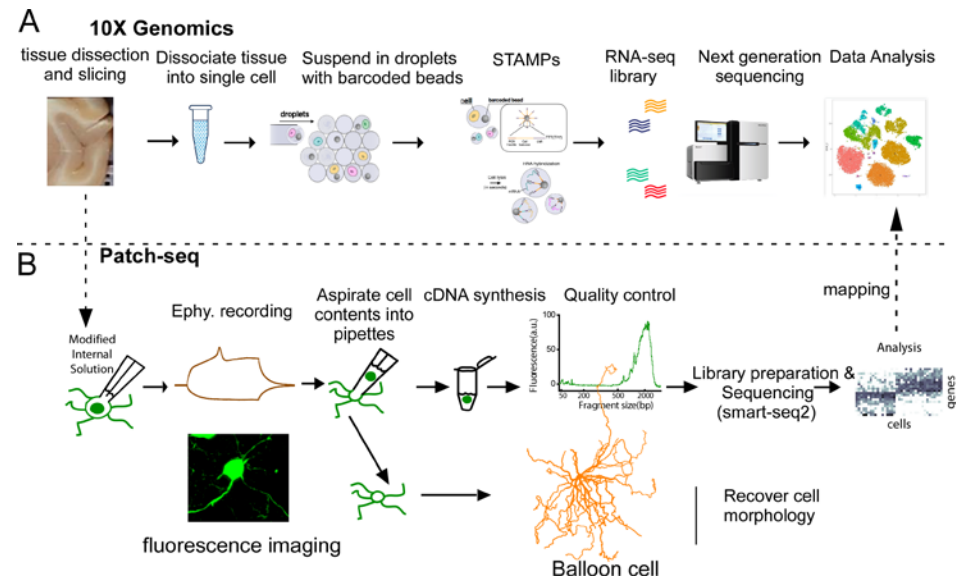


**Fig.4. Combination of 10X Genomics drop-seq and Patch-seq protocols to correlate electrophysiology, morphology, and transcriptome of each cell type.** (A) Droplet-based scRNA-seq of a large number of single cells from cortical tube (CT) identifies all transcriptomic cell types that comprise CT and specific genetic markers for each cell type. (B). Patch-seq approach to link each transcriptomic cell type to their morphology and electrophysiological properties. The cell types such as balloon cells in CT can be identified first by their unique soma (balloon-like cell shape) in the human brain slices, then by their unique firing properties and morphology (by fluorescence imaging post hoc biocytin staining). Their transcriptomes will be mapped to the transcriptomic atlas generated from 10X Genomics scRNA-seq to identify the transcriptimoc cluster(s) corresponding to balloon cells.

***Mapping the transcriptome collected by Patch-seq to the reference transcriptomic atlas from the droplet-based approach:*** We will follow our previous mapping methods to map the transcriptomic data across two scRNA-seq protocols (Scala et al., 2018; Cadwell et al., 2019) (Fig 4A, B). Using the count matrix of reference scRNA data from dissociated cells, we select 3000 "most variable" genes as described previously (Kobak et al., 2018). We then log-transformed all counts with log2(x+1) transformation and averaged the log-transformed counts across all cells in each of the identified clusters, to obtain reference transcriptomic profiles of each cluster (N×3000 matrix). Out of these 3000 genes, 2575 are present in the mm10 reference genome that we used to align reads in our patch-seq data. We apply the same log2(x+1) transformation to the read counts of Patch-seq cells, and for each cell computed Pearson correlation across the 2575 genes with all clusters. Each cell is assigned to the cluster to which it has the highest correlation.

We have performed the droplet-based scRNA-seq on our first cortical tube sample collected from a 2-year-old TSC patient. We profiled the transcriptomes of 6,381 cells from this cortical tube sample. Analysis of the sequence reads revealed on average ~110,000 uniquely detected transcripts from ~2300 genes for each cell. After filtering out cells with limited numbers of detected genes, our dataset contained 5,639 cells.

From these cells, we identified 20 potential cellular clusters (Fig.5A). We use the expression patterns of known marker genes to identify major cell-types (neuron, glia, and microglia et. al) (Maragakis et al., 2004; Marques et al., 2016; Crotti and Ransohoff, 2016). We separated neuronal from non-neuronal clusters based on pan-neuronal marker Snap25 (encoding synaptosome-associated protein 25). Interestingly, several clusters (circled) express both neuronal markers (SNAP5, SLC1A1) and non-neuronal markers (SLC1A2 and SLC1A3) (Fig 5B), which may be specific to CT. In addition, these clusters may be inhibitory clusters, since they were void of Slc17a7 (VgluT1), an excitatory neuronal marker genes (Fig 5B).

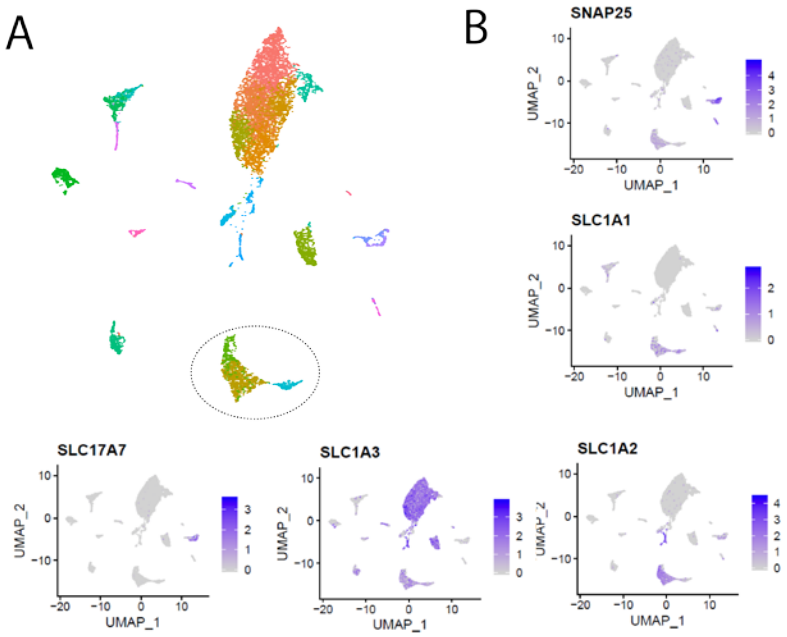


**Fig.5. scRNA-seq of 5,639 cells from cortical tube of a human TSC patient reveals all** molecular cell types in this pathological tissue. A) Two-dimensional UMAP visualization showing the distribution of all cells from CT. B) Using the known cell type-specific markers, we assign identities to each cluster and differentiate neuronal clusters (enriched with Snap25) from non-neuronal clusters (oligodendrocytes, astrocyte, microglia et al). Interestingly, several clusters (circled) express both neuronal marker (SNAP5, SLC1A1) and non-neuronal markers (SLC1A2 and SLC1A3)

While this clustering analysis and putative genetic marker for each cell type are very preliminary due to low cell numbers, these preliminary results do provide proof-of-principle for CT profiling with 10x Genomics scRNA-seq. We have collected more human tissue samples from control and TSC patients for more 10x Genomics scRNA-seq, aiming at generating a comprehensive taxonomy of molecular cell types in CT, as well as a census of cell type in control tissue. This molecular cell atlas will be used as a comprehensive reference against which to compare the Patch-seq data to identify those clusters corresponding to balloon cells and cytomegalic interneurons (Fig 4).

We have collected 10 Patch-seq samples from balloon cells and a few samples from excitatory neurons so far (Fig.5). We need to collect Patch-seq samples from cytomegalic neurons, particularly cytomegalic interneurons in the next reporting period. Then we will use the method described above to identify the transcriptomic cluster(s) corresponding to each abnormal cell type, especially balloon cell and cytomegalic interneurons, and understand the gene expression profiles that underlie their unique morphology, electrophysiology, and connectivity.

**What opportunities for training and professional development has the project provided?**

*If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency.*

*Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.*

**How were the results disseminated to communities of interest?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.*

*Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

***4.*** **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

**What was the impact on the development of the principal discipline(s) of the project?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

**What was the impact on other disciplines?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

**What was the impact on technology transfer?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

**What was the impact on society beyond science and technology?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

*hat*

**Actual or anticipated problems or delays and actions or plans to resolve them**
*Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

**Changes that had a significant impact on expenditures**
*Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
*Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

**Significant changes in use or care of human subjects**

No

**Significant changes in use or care of vertebrate animals**

No

**Significant changes in use of biohazards and/or select agents**

No

**6. PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  *Report only the major publication(s) resulting from the work under this award.*

  **Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  *Nothing to Report*

**Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

*Nothing to Report*

**Other publications, conference papers and presentations**. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

1. A poster was presented in a local conference,
2. A seminar was given to local neurosurgeons.

- **Website(s) or other Internet site(s)**

*List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.*

*Nothing to Report*

- **Technologies or techniques**

*Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.*

*Nothing to Report*

- **Inventions, patent applications, and/or licenses**
  *Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.*

| |
|---|
| *Nothing to Report* |

- **Other Products**
  *Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:*
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

| |
|---|
| *Nothing to Report* |

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

**What individuals have worked on the project?**
*Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".*

| | |
|---|---|
| *Name:* | *Xiaolong Jiang* |
| *Project Role:* | *PI* |
| *Researcher Identifier (e.g. ORCID ID):* | *000-001-8066-1383* |
| *Nearest person month worked:* | *1* |
| *Contribution to Project:* | *Dr. Jiang has performed slice electropgysiology ,* |

*Funding Support:* *R01 MH120404; U19 MH114830; R01 MH109556; IARPA #D16PC0003*

*Name:* *Andrew McKinney*
*Project Role:* *Gradute student*
*Researcher Identifier (e.g. ORCID ID):* *N/A*
*Nearest person month worked:* *1*
*Contribution to Project:* *Mr. McKinney has performed slice electropgysiology and connectivity study, and data analysis.*
*Funding Support:* *T32 training grant: T32 MH 312008*

*Name:* *Junzhan Jing*
*Project Role:* *Gradute student*
*Researcher Identifier (e.g. ORCID ID):* *000-003-4647-0932*
*Nearest person month worked:* *1*

*Contribution to Project:* *Dr. Jing has performed slice electropgysiology and connectivity study, single-cell RNA-sequencing, and data analysis.analysis,*
*Funding Support:* *NIH R01 MH120404, R01 MH109556*

*Name:* *Qianqian Ma*
*Project Role:* *Technician*
*Researcher Identifier (e.g. ORCID ID):* *N/A*
*Nearest person month worked:* *1*

*Contribution to Project:* *Mrs. Ma has performed tissue collection and help with slice electrophysiology*
*Funding Support:* *NIH R01 MH120404*

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.*

The changes:

R01 MH120404 was awarded to PI on July 1 2019

R01 MH122169 awarded to PI on April 1 2020

**What other organizations were involved as partners?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.*

*Provide the following information for each partnership:*
*Organization Name:*
*Location of Organization: (if foreign location list country)*
*Partner's contribution to the project (identify one or more)*

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

| |
|---|
| *Nothing to Report* |

**8. SPECIAL REPORTING REQUIREMENTS**

**COLLABORATIVE AWARDS:** *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

**QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

**9. APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*